

The *Asplenium trichomanes* Complex in the United States and Adjacent Canada

ROBBIN C. MORAN*

The Maidenhair Spleenwort, *Asplenium trichomanes* L., is a delicate, evergreen fern that grows in rock crevices or, less frequently, on talus slopes. The species is wide ranging, occurring in North America, Europe, Australia, New Zealand, and Asia. Since Manton (1950) reported the existence of diploid and tetraploid cytotypes in European populations of *A. trichomanes*, both cytotypes and their sterile triploid hybrids have also been found in North America (Britton, 1953; Wagner & Wagner, 1966). The diploid and tetraploid cytotypes that occur in North America have been named, the diploid as subsp. *trichomanes* and the tetraploid as subsp. *quadrivalens* D. E. Meyer emend. Lovis. This paper examines for the first time the occurrence and relative abundance of all three *A. trichomanes* cytotypes in the United States and Canada, and compares this information to what is known about this species complex in Europe.

METHODS

Spore size was used to determine cytotype. To determine the validity of this approach, a pilot study was undertaken that involved an examination of spore length from 30 herbarium specimens that were cytologically determined by Drs. F. S. and W. H. Wagner. Tetraploids consistently had much larger exospore diameters than the diploids (Fig. 1). In fact, differences in spore size were so obvious that laborious measurements of spore size were unnecessary to distinguish diploids from tetraploids.

The total number of plants examined for spore size was 115 (1725 spores) for the diploids, and 52 (810 spores) for the tetraploids. Triploid plants were recognized by the presence of aborted spores. Thus, for this study, the presumed cytotype was extrapolated from spore size or spore abortion rather than direct chromosome counts.

Over 700 herbarium specimens were examined from the following institutions: EIU, F, ILL, ILLS, ISM, MICH, MO, NCU, NHA, RM, SIU, TENN, VDB, and WTU. For each specimen examined, a permanent spore slide was made using Hoyer's Solution as a mounting medium and sealing the cover slip with clear nail polish enamel. After the slides had been examined, they were placed in a paper envelope and glued to the herbarium sheet for permanent reference; the specimens were annotated indicating the presumed cytotype. Locality data of individual collections are available from the author.

RESULTS AND DISCUSSION

The mean spore size for diploid plants was 29.4 μm ($s=2.3$) and for tetraploid plants 41.0 μm ($s=2.6$). These results support those of Lovis (1964), who found similar differences in mean spore size of *A. trichomanes* cytotypes in Europe.

*Illinois Natural History Survey, Natural Resources Bldg., 607 E. Peabody Dr., Champaign, IL 61820.

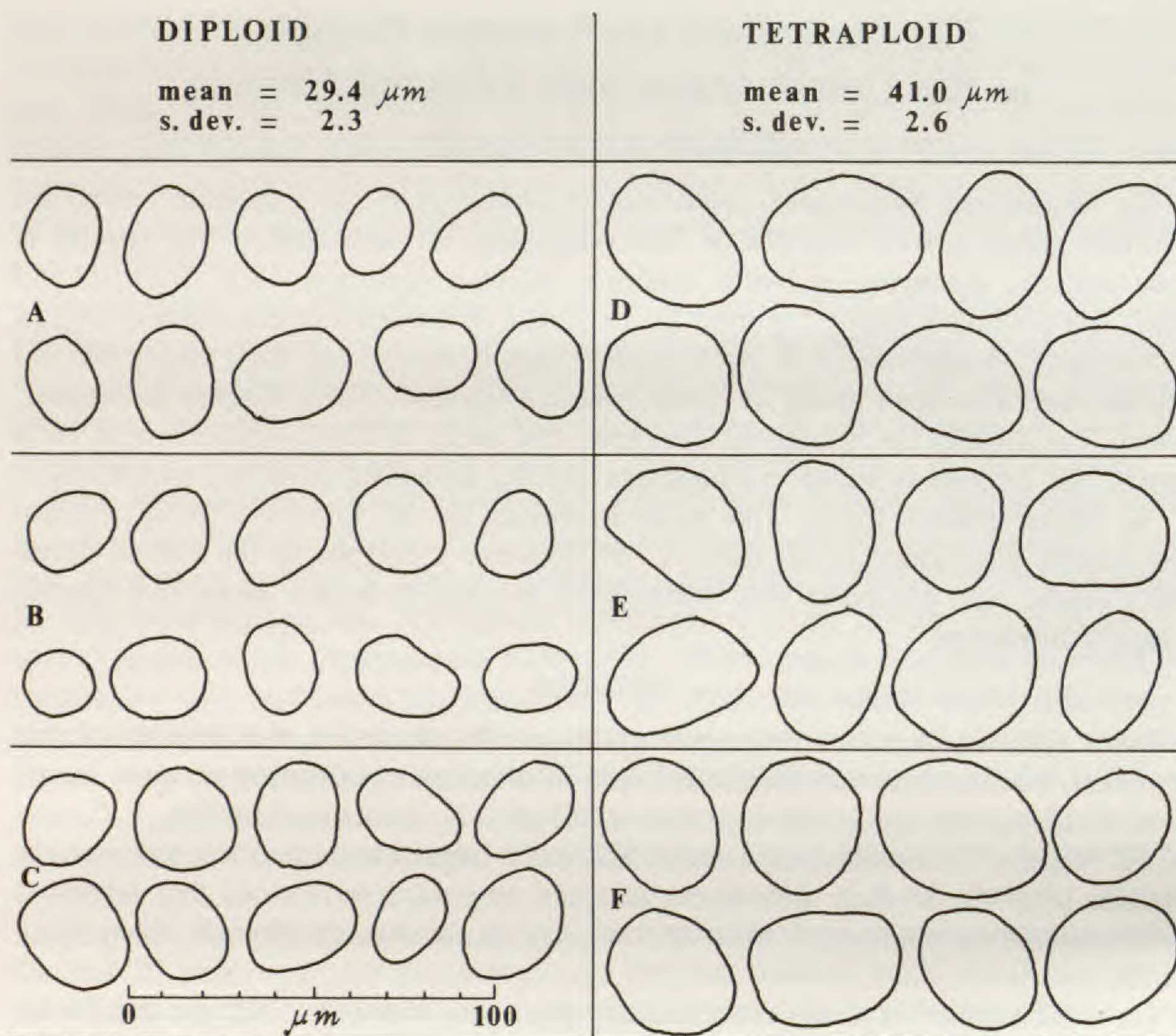


FIG. 1. Outlines of random spore samples mounted in Hoyer's Solution. A. Virginia, Giles County, limestone along Sinking Creek, 1 mile north of Newport (specimen cytologically det.), *W. H. Wagner 64113* (MICH). B. Illinois, Hardin County, sandstone outcrop in Blind Hollow, southeast of Lamb, *R. A. Evers 20091* (ILLS). C. Kansas, Chatauqua County, 3.5 miles east and 4 miles north of Sedan, wooded sandstone canyon, *L. Hauser 3134* (NCU). D. Same location as A, specimen cytologically det., *W. H. Wagner 64115* (MICH); E. Virginia, Loudoun County along Potomac River, north-northeast of Leesburg, *H. E. Ahles 61196* (NCU). F. Canada, British Columbia, limestone cliffs at head of Cumshewa Inlet near Moresby logging camp, Moresby Island, part of Queen Charlotte Islands, *J. A. Calder & R. L. Taylor 36254* (NCU).

European workers (Jermy et al., 1978; Jermy & Page, 1980; Lovis, 1964) have found that plants of diploid and tetraploid *A. trichomanes* can usually be distinguished without recourse to micro-characters. However, this is extremely difficult, if not impossible, to do satisfactorily from herbarium specimens; thus the reliance in this study on the more constant character of spore size. The morphological characters listed in the key given below are best observed in living plants. Lovis (1964) has published shadow diagrams that illustrate morphological differences in well developed fronds of diploid and tetraploid plants. North American pteridologists would do well to become familiar with the characters listed in the key below

and to pay close attention as to their applicability in living specimens of North American plants.

Plants mostly of non-calcareous rocks; rhizome scales up to 3 mm long; stipe relatively thin, shiny coppery or bronze red; fronds arching upwards and outwards, away from rock faces; pinnae mostly alternate, suborbicular, more distant, thinner, set more oblique to the rachis, the basiscopic margin often auriculate, pinnae usually shed from the rachillae during the first winter, the rachillae retained during first winter, except in exposed habitats; sori 4-6(9), up to 2 mm long; indusia delicate, not conspicuous, spores generally 29-36 μm long; guard cells generally 38-43 μm long; plants diploidsubsp. *trichomanes*

Plants of calcareous rocks; rhizome scales up to 5 mm long; stipe relatively thicker, blackish-brown; fronds closely adpressed to rock faces, sinuously spreading in all directions; pinnae mostly opposite, oblong, more crowded, thicker, nearly perpendicular to the rachis, the basiscopic margin rarely auriculate; pinnae usually retained during the first winter, the rachillae shed with, or soon after, the pinnae; sori 4-9(12), up to 3 mm long; indusia thicker, conspicuous; spores generally 34-43 μm long; guard cells generally 41-49 μm long; plants tetraploidsubsp. *quadrivalens*

The diploid is the most abundant and widespread cytotype, constituting 87% of the specimens examined. Specimens of this cytotype from the southwestern United States (*Fig. 2*) are from high altitudes in isolated mountain ranges, such as the Rincon and Santa Catalina mountains in southern Arizona, where several collections were made between 2500 and 3000 meters elevation. Diploids are found primarily on acid rock types such as sandstone, basalt, olivine, etc. However, 12 specimens were collected from limestone. There was no discernable geographic pattern to the diploid plants found on limestone.

Tetraploid plants are primarily northern in distribution, being common in the middle Great Lakes and New England regions (*Fig. 3*). Approximately 12% of the 700 specimens examined were tetraploid. This cytotype appears to be an obligate calciphile, as every specimen examined was collected from limestone habitats. In Europe and Australia, Lovis (1964) also found the tetraploid cytotype confined to calcareous habitats. Wagner and Wagner (1966) studied a population of *A. trichomanes* on limestone cliffs along Sinking Creek, about 1 mile north of Newport, in Giles County, Virginia. From a random sample of 89 plants, 85 proved to be tetraploid, 3 triploid, and 1 diploid. The abundance of tetraploids at this site emphasizes the calciphilous nature of this cytotype. One may wonder why the tetraploid is not more widespread, as suitable limestone habitats certainly exist in many localities outside its main range, such as the Ozarks of Missouri.

The distribution of diploid and tetraploid plants in the Great Lakes region also graphically demonstrates their substrate preferences. The tetraploid is restricted to the Niagara escarpment of Silurian limestone, which forms an arc running from Door County, Wisconsin, across the lower northern peninsula of Michigan to Manitoulin Island, the Bruce Peninsula, and thence southeastward to Niagara Falls in New York. Southwest of Door County, Wisconsin, and southeast of Niagara Falls, the Niagara limestone escarpment becomes gradually covered by glacial tills and is not exposed. The great number of herbarium specimens from locations on the Niagara escarpment suggests that the tetraploid is fairly common there. In contrast, the diploid in the Great Lakes region occurs primarily on Precambrian igneous and metamorphic rocks, such as those that form the Keweenaw peninsula and the escarpments along the north shore of Lake Superior.



2



3

FIG. 2. The distribution of diploid *Asplenium trichomanes* in the United States and southern Canada.
FIG. 3. The distribution of tetraploid (dot) and triploid (triangle) *Asplenium trichomanes* in the United States and southern Canada.

The triploid is the rarest cytotype; only seven collections (1.0%) were seen from the eastern United States (Fig. 3). The triploid occurs sporadically where the diploid and tetraploid populations overlap in the eastern Appalachians. Limestone was given as the rock type in all cases where herbarium labels provided such information.

It is important to note, since many triploid plants are apogamous, that there was no indication of large, globose, unreduced spores in any of the triploids examined. Furthermore, in the above-mentioned population of *A. trichomanes* studied by Wagner and Wagner (1966), the triploids appeared to be the result of *in situ* hybridization from diploid and tetraploid plants occurring at the site.

Figures 2 and 3 show only the main range of *A. trichomanes* in the United States and southern Canada, but *A. trichomanes* also occurs sporadically eastward in Canada to Newfoundland (Cody, 1968) and northward as isolated occurrences in the western cordillera of Alberta and British Columbia to southeastern Alaska at ca. 58° north latitude (Hultén, 1968; Scoggan, 1978). I have not seen specimens from these extremes of the species range. *Asplenium trichomanes* also occurs on limestone rocks on the Queen Charlotte Islands of British Columbia. All collections examined from this locality were tetraploid, which agrees with the cytological determinations of Taylor and Mulligan (1968). The disjunct *A. trichomanes* individual reported by Cody (1968) from Newfoundland is probably a diploid since it occurred on serpentine rocks.

It is important to note that hexaploid plants are known from Madeira, Australia, and New Zealand. However, only in New Zealand is the hexaploid a prevalent cytotype (Lovis, 1977). No evidence of hexaploid plants was found in this study, based on spore size, although their occurrence in North America could be expected from chromosome doubling of a triploid plant.

No apparent morphological differences were found between European and North American *A. trichomanes*. Indeed, the fact that the same rock habitat differences exist between the diploid and tetraploid cytotypes in both Europe and North America suggests that they are the same taxa. Furthermore, no specimens of *A. trichomanes* subsp. *inexpectans* Lovis, a diploid subspecies known only from central and southeastern Europe, were found among diploids in North America which grew on limestone. This subspecies is similar to subsp. *trichomanes*, but occurs only on calcareous rocks and has a slightly different frond morphology (Lovis, 1964, fig. 2).

Unlike other *Asplenium* species in the eastern United States, *A. trichomanes* rarely hybridizes and its genome is not implicated in any widespread allopolyploid taxa. In Europe, however, a diploid form of *A. trichomanes* has hybridized with diploid *A. viride* Huds. giving rise to the fertile allotetraploid known as *A. adulterinum* Milde., which is widely dispersed in northern and central Europe (Lovis, 1977). Four sterile, interspecific hybrids involving *A. trichomanes* have been found in the eastern United States, these are: *A. ×clermontae* Sim (*A. ruta-muraria* × 4x *A. trichomanes*), *A. ×virginicum* Maxon (supposedly *A. platyneuron* × 2x *A. trichomanes*), *×Asplenosorus herb-wagneri* (Taylor & Mohlenbrock) Mickel (*×A. pinnatifidum* × 2x *A. trichomanes*) and *×A. shawneensis* R. C. Moran (2x *A. trichomanes* × *Camptosorus rhizophyllus*). Each of these hybrids is exceedingly rare, being known from only one or two localities. It

is extraordinary that $\times A. shawneensis$ has been found only once, especially since its two parents often grow side-by-side throughout much of the well botanized eastern United States (Moran, 1981).

Certain aspects of the origin of tetraploid *A. trichomanes* remain enigmatic. The tetraploid cytotype appears to be of autopolyploid constitution, as shown by the high levels of autosyndetic chromosome pairing seen in various hybrids (Lovis et al., 1966; Lovis & Reichstein, 1969; Vida, 1970) and an experimentally produced polyhaploid sporophyte (Bouharmont, 1972). However, Lovis (1977) postulates that the origin of tetraploid *A. trichomanes* is polyphyletic, in that tetraploid forms may have arisen by different means at different times and in different parts of the world. The North American tetraploid cytotype may have originated by direct autopolyploidy from diploid forms or by hybridization between diploid subspecies, followed by chromosome doubling, producing intersubspecific autopolyploidy (Lovis, 1977, p. 369). Whatever the case may be, the calciphilous nature of the tetraploid must be taken into account when postulating its polyploid origin from a diploid ancestor. The tetraploid's remarkable habitat preference for limestone is not what would be expected from direct autopolyploidy of a diploid taxon that predominantly occurs on acid rocks. Perhaps the North American tetraploid arose via autopolyploidy from a calciphilous diploid; or perhaps this diploid, calciphilous race hybridized with the more common acidic rock race, with subsequent allopolyploidy, and in so doing imparted a dominant adaptive gene complex enabling the tetraploid to thrive in calcareous habitats. Although many techniques seem inadequate to resolve such a potentially complex evolutionary history, an analysis of isozyme variation from populations throughout the entire range of the complex might yield interesting results.

I would like to thank the following for their help with various aspects of the study: Drs. Almut Jones, James H. Peck, W. Carl Taylor, Charles Werth, Florence S. Wagner, and Warren H. Wagner, Jr. Special thanks is given to Dr. Kenneth R. Robertson of the Illinois Natural History Survey for providing the writer with research time and encouragement for this project.

LITERATURE CITED

- BOUHARMONT, J. 1972. Meiosis and fertility in apogamously produced diploid plants of *Asplenium trichomanes*. *Chromosomes Today* 3:253-258.
- BRITTON, D. M. 1953. Chromosome studies on ferns. *Amer. J. Bot.* 40:575-583.
- CODY, W. J. 1968. *Asplenium trichomanes* new to Newfoundland. *Amer. Fern J.* 58:179-180.
- HULTÉN, E. 1968. *Flora of Alaska and Neighboring Territories*. Stanford Univ. Press, Stanford, CA.
- JERMY, A. C., H. R. ARNOLD, L. FARRELL, and F. H. PERRING. 1978. *Atlas of Ferns of the British Isles*. Botanical Society of the British Isles and British Pteridological Society, London.
- , and C. N. PAGE. 1980. Additional field characters separating the subspecies of *Asplenium trichomanes* in Britain. *Brit. Fern Gaz.* 12:112-113.
- LOVIS, J. D. 1964. The taxonomy of *Asplenium trichomanes* in Europe. *Brit. Fern Gaz.* 9:147-160.
- . 1977. Evolutionary patterns and processes in ferns. Pp. 229-415 in R. D. Preston and H. W. Woolhouse (eds.). *Advances in Botanical Research*, vol. 4. Academic Press, New York.
- , H. MELZER, and T. REICHSTEIN. 1966. *Asplenium* \times *stiriicum* D. E. Meyer emend. und *A.* \times *aprutianum* hybr. nov., die zwei *Asplenium lepidium* \times *trichomanes*-Bastarde. *Bauhinia* 3:87-101.

- MANTON, I. 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge Univ. Press, Cambridge.
- MORAN, R. C. 1981. \times *Asplenosorus shawneensis*: a new natural fern hybrid between *Asplenium trichomanes* and *Camptosorus rhizophyllus*. Amer. Fern J. 71:85-89.
- SCOGGAN, H. J. 1978. The Flora of Canada, Part 2. Pteridophyta, Gymnospermae, Monocotyledoneae. Canad. Natl. Mus. Sci. Publ. Bot. 7:93-545.
- TAYLOR, R. L. and G. A. MULLIGAN. 1968. Flora of the Queen Charlotte Islands, Part 2. Cytological Aspects of the Vascular Plants. Canad. Dept. Agr. Monogr. 4, Part 2.
- VIDA, G. 1970. The nature of polyploidy in *Asplenium ruta-muraria* L. and *A. lepidium* C. Presl. Caryologia 23:525-547.
- WAGNER, W. H., Jr. and F. S. WAGNER. 1966. Pteridophytes of the Mountain Lake Area, Giles Co., Virginia: biosystematic studies, 1964-1965. Castanea 31:121-140.

REVIEW

"ILLUSTRATION OF THE PTERIDOPHYTES OF JAPAN, VOLUME I," by Satoru Kurata and Toshiyuki Nakaike. University of Tokyo Press. 628pp. 1979. Yen 50,000.—This book represents the first volume of a series of works devoted to illustrating and mapping the more than 600 species of Japanese (including the Ryu-Kyu and Bonin Islands) pteridophytes. This volume treats 100 species from a variety of families and includes large segments, though regrettably not all, of many genera such as *Dryopteris*, *Polystichum*, and *Pteris*. The treatment of individual species includes photographs depicting habit, line drawings of whole plants as well as details of sori, venation, cuticle, scales, and rhizome anatomy, a short description of the species, and an enumeration of specimens examined with a full page grid-map of Japan showing their location. The entire volume is beautifully produced with excellent paper and binding, something of which more American publishers ought to be envious.

Although the descriptive material is entirely in Japanese (Latin names are included), the illustrations and detailed distributions will be quite useful to American botanists, not only pteridologists, but phytogeographers and ecologists as well. It is hard to ignore, for instance, the close resemblance of our *Dryopteris ludoviciana* with *D. tokyoensis* after examining the photograph and illustration of the latter. Although it is unlikely that many individuals will be able to purchase this book, they should certainly encourage their institutional libraries to do so.—*R. Cranfill, Department of Botany, University of California, Berkeley, CA 94720.*